Personal $PM_{2.5}$ Exposure and Markers of Oxidative Stress in Blood

Mette Sørensen,[1] Bahram Daneshvar,[2] Max Hansen,[2] Lars O. Dragsted,[2] Ole Hertel,[3] Lisbeth Knudsen,[1] and Steffen Loft[1]

[1]Institute of Public Health, University of Copenhagen, Denmark; [2]Institute of Food Safety and Nutrition, Søborg, Denmark; [3]National Environmental Research Institute, Roskilde, Denmark

Ambient particulate air pollution assessed as outdoor concentrations of particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$) in urban background has been associated with cardiovascular diseases at the population level. However, the significance of individual exposure and the involved mechanisms remain uncertain. We measured personal $PM_{2.5}$ and carbon black exposure in 50 students four times in 1 year and analyzed blood samples for markers of protein and lipid oxidation, for red blood cell (RBC) and platelet counts, and for concentrations of hemoglobin and fibrinogen. We analyzed protein oxidation in terms of γ-glutamyl semialdehyde in hemoglobin (HBGGS) and 2-aminoadipic semialdehyde in hemoglobin (HBAAS) and plasma proteins (PLAAS), and lipid peroxidation was measured as malondialdehyde (MDA) in plasma. Median exposures were 16.1 μg/m^3 for personal $PM_{2.5}$ exposure, 9.2 μg/m^3 for background $PM_{2.5}$ concentration, and 8.1 × 10^{-6}/m for personal carbon black exposure. Personal carbon black exposure and PLAAS concentration were positively associated ($p < 0.01$), whereas an association between personal $PM_{2.5}$ exposure and PLAAS was only of borderline significance ($p = 0.061$). A 3.7% increase in MDA concentrations per 10 μg/m^3 increase in personal $PM_{2.5}$ exposure was found for women ($p < 0.05$), whereas there was no significant relationship for the men. Similarly, positive associations between personal $PM_{2.5}$ exposure and both RBC and hemoglobin concentrations were found only in women ($p < 0.01$). There were no significant relationships between background $PM_{2.5}$ concentration and any of the biomarkers. This suggests that exposure to particles in moderate concentrations can induce oxidative stress and increase RBCs in peripheral blood. Personal exposure appears more closely related to these biomarkers potentially related to cardiovascular disease than is ambient $PM_{2.5}$ background concentrations. *Key words:* **carbon black, fibrinogen, hemoglobin, lipid peroxidation, particulate matter, platelets, protein oxidation, red blood cells.** *Environ Health Perspect* **111:161–165 (2003). [Online 31 October 2002]**

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Epidemiologic studies have shown that particulate air pollution in the urban environment is associated with an increased risk in human mortality and morbidity, especially that caused by cardiopulmonary diseases (Dockery et al. 1993; Peters et al. 2001; Pope et al. 2002). One of the proposed mechanisms is increased oxidative stress. This is assumed to be mediated partly by particle-induced inflammation in the lungs, causing macrophages to release reactive oxygen species (ROS), and partly by transition metals on the particle surface capable of generating ROS through the Fenton reaction (Donaldson et al. 1997; Li et al. 1997). The atherosclerotic process is thought to involve oxidation of low-density lipids, and a recent intervention study has shown that progression of arteriosclerosis can be reduced in heavily smoking men by supplementing with a combination of antioxidant vitamins C and E (Salonen et al. 2000). Long-term exposure to particles could thus possibly increase the risk of developing atherosclerotic plaques through increased oxidation of low-density lipids. Other possible mechanisms are short-term effects such as increased blood viscosity due to lung inflammation (Peters et al. 1997) or a direct effect of inhaled particles on the concentration of several hematologic parameters such as platelets and red blood cells (RBCs) (Salvi et al. 1999; Seaton et al. 1999). There is increasing evidence that abnormalities in blood rheology are related to various cardiovascular diseases (Koenig and Ernst 1992). A large epidemiologic study found blood viscosity to predict cardiovascular events as efficiently as low-density lipoproteins (LDL), cholesterol, and blood pressure (Lowe et al. 1997).

In most studies assessing particulate air pollution and health, outdoor monitoring of the urban background of particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$) or particulate matter ≤ 10 μm in diameter (PM_{10}) concentrations have been used as indicators for particle exposure. However, people spend most of their time indoors, and indoor sources of air pollutants are numerous (Jenkins et al. 1992; Ozkaynak et al. 1996). This makes risk assessment based mainly on outdoor measurements uncertain because the outdoor particle concentrations used to estimate exposure in health studies may not reflect true population exposures to particulate matter (Ozkaynak et al. 1996). Monitoring personal exposure in relation to adverse health effects may be difficult. However, by means of biomarkers mechanistically related to relevant health effects, it may be possible to assess relevant exposure to particulate matter and the involved sources.

Our aim in this study was to examine the effect of personal exposure to fine particles on levels and damage to several components of the blood. We investigated this in 50 students living and studying in central Copenhagen by measuring the personal $PM_{2.5}$ exposure in 2-day periods followed by collection of blood samples. This was repeated four times in 1 year. We analyzed the blood samples for markers of protein and lipid oxidation, for RBCs and platelet counts, and for concentrations of hemoglobin and fibrinogen.

Materials and Methods

Experimental design. We measured personal exposure to $PM_{2.5}$ and carbon black in 50 students living and studying in central Copenhagen. To account for seasonal variation, we repeated the measurements four times in one year. Five-week measuring campaigns were conducted in November 1999 (autumn), January–February 2000 (winter), April–May 2000 (spring), and August 2000 (summer). Measurements were conducted over 2-day periods for each subject, with five subjects being monitored from Monday morning to Wednesday morning and five subjects being monitored from Wednesday morning to Friday morning. In each 2-day monitoring period, urban background concentrations of $PM_{2.5}$ were also measured on a roof of a building on the Copenhagen University campus.

The subjects were recruited through a notice in the local university paper. They were all nonsmokers, living and studying in central Copenhagen, and were between 20 and 33 years old, with a median age of 24 years. There was an even distribution of males and females. Not all of the 50 subjects could participate in all four seasons. Therefore, new subjects were recruited so that 50 subjects participated in each season. In all, 68 subjects participated, of whom 31 subjects participated in all four campaigns (corresponding to 124 measurements), 12 subjects participated

Address correspondence to S. Loft, Institute of Public Health, c/o Department of Pharmacology, The Panum Institute, Room 18-5-32, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. Telephone +45 35327649. Fax +45 35327610. E-mail: s.loft@pubhealth.ku.dk

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in three campaigns (corresponding to 36 measurements), 10 subjects participated in two campaigns (corresponding to 20 measurements), and 15 subjects participated in only one campaign (corresponding to 15 measurements). Altogether, we collected 195 measurements, which were all included in the subsequent statistical analysis. Morning blood samples were collected at the end of each 2-day campaign. The local ethics committee approved the study protocol, and the subjects gave written informed consent before entry into the study.

Air sampling and analysis. The particles were sampled with a system from the International Gravity Bureau (BGI, Toulouse, France) (Kenny and Gussman 1997), a KTL $PM_{2.5}$ cyclone developed for the European EXPOLIS study (Jantunen et al. 1998), a BGI400 pump (BGI Inc., Waltham, MA, USA) (flow 4 L/min), and a battery for 48-hr operation. The equipment for personal sampling was placed in a backpack, which the subjects carried or placed nearby when they were indoors. Sampling was done on 37-mm Teflon filters (Biotech Line, Lynge, Denmark). Before and after sampling the filters were weighed on a Micro weight MT5 from Mettler-Toledo (Glostrup, Denmark) after conditioning for 24 hr in the laboratory. We determined the detection limit to about 26 µg, defined as three times the standard deviation on the blank, which typically amounted to 5–20%. On the basis of eight parallel measurements, repeated six times, the coefficient of variation was calculated as 14.4%.

We measured the reflectance (carbon black) of the $PM_{2.5}$ filters on a Model 43 Smokestain Reflectometer (Diffusion Systems LTD, London, UK). On each filter the reflectance was measured with triple determinations in five different spots. The 15 measurements were averaged and transformed into the absorption coefficient (a, per meter) using the following formula (ISO 1993):

$$a = (A/2V) \times \ln(R_0/R),$$

where A is the area of the stain on the filter paper (square meters), V the volume sampled (cubic meters), R the intensity of reflected light from the exposed filter, and R_0 is the intensity of reflected light from a clean filter. The coefficient of variation was 22.2% on the basis of eight parallel measurements repeated five times. Three carbon black measurements were below the detection limit of 0.01×10^{-6}/m. To include these measurements in a logarithmic model, we gave them the value of 0.007×10^{-6}/m, estimated according to the formula: detection limit/$\sqrt{2}$, suggested by Hornung and Reed (1990).

Hematologic measurements. RBC and platelet count as well as hemoglobin and fibrinogen concentrations were measured at the Department of Clinical Biochemistry, Copenhagen University Hospital. After venous puncture, blood samples for determination of hemoglobin concentration and RBC and platelet counts were collected in sodium citrate tubes (Becton Dickinson Vacutainer Systems, Plymouth, UK). The blood samples were then stored at room temperature until they were measured within 3 hr on a Sysmex SE 9000 analyzer (Sysmex, Long Grove, IL, USA). For measurement of fibrinogen concentration, we collected the blood samples in K_3EDTA tubes (Becton Dickinson Vacutainer Systems) and analyzed them within 3 hr on an ACL Futura coagulometer (Beckman Coulter, Fullerton, CA, USA). We used a commercially available kit (ABX Diagnostics, Montpellier, France) to measure concentrations of plasma protein. Plasma protein concentration was a significant predictor of the hemoglobin concentration ($p < 0.05$) and a borderline predictor of the RBC count ($p = 0.10$). This indicates that dilution and/or concentration of the blood has an effect on the hematologic parameters measured. To eliminate this source of error, we conducted all the analyses on data corrected for plasma protein (hematologic parameter/gram plasma protein).

Protein and lipid oxidation. For analysis of protein and lipid oxidation biomarkers, the blood samples were collected in sodium heparin tubes (Termo Venoject glass tubes, Leuven, Belgium). Plasma and RBCs were collected after centrifugation (1,500*g*) and stored at –80°C until analysis. We assessed protein oxidation by the concentration of γ-glutamyl semialdehyde in hemoglobin (HBGGS) and 2-aminoadipic semialdehyde in hemoglobin (HBAAS) and in plasma proteins (PLAAS), as described previously (Daneshvar et al. 1997). Analyses were done in single measurements. For the PLAAS and HBAAS measurements, the intraday coefficient of variation (CV) is 2%, and the interday CV is 7%. For HBGGS the interday CV is 2%, and the interday CV is 8%. Total MDA in plasma was determined by HPLC in doublet measurements as previously described (Lauridsen and Mortensen 1999). The MDA measurements have been found to have an interday CV of 6% and an interday CV of 11%. In all four analyses we included an internal standard in every run, and if this internal standard differed more than 10% compared to the expected value, the run was repeated.

Statistics. All statistical analyses were done using SAS software (version 8e; SAS Institute, Cary, NC). We used mixed model repeated-measures analysis (Proc MIXED) to describe concentrations of the hematologic parameters (RBC, hemoglobin, platelets and fibrinogen) as well as oxidation of protein (HBGGS, HBAAS and PLAAS) and lipid (MDA) as a function of various predictors. As explanatory variables (predictors) we included season, average outdoor temperature, sex, and, in three separate models for each dependent variable, the exposure markers personal $PM_{2.5}$ exposure, personal carbon black exposure, and background $PM_{2.5}$ concentration. The interaction between the exposure marker in question and sex was included in each model to analyze whether sex modified a possible association between the exposure marker and the dependent variable. Subject nested in sex was included as a random factor to

Table 1. Hematologic parameters and exposure markers overall and by sex.

	All		Men			Women			
	Median	Q25–Q75	Median	Q25–Q75	No.	Median	Q25–Q75	No.	*p*-Value[a]
RBC count (× 10^9/g protein)	61.5	56.8–67.7	65.4	60.9–69.2	96	58.0	55.5–61.5	91	< 0.0001
Hemoglobin (µmol/g protein)	114	106–126	122	112–128	96	109	103–116	91	< 0.0001
Platelet count (× 10^6/g protein)	3.14	2.62–3.80	2.77	2.44–3.13	95	3.57	3.20–4.16	91	< 0.0001
Fibrinogen (nmol/g protein)	130	99–159	103	86–130	67	153	130–187	70	< 0.0001
HBGGS (pmol/mg protein)	38.0	32.0–45.1	35.0	29.0–41.0	95	41.6	35.7–51.0	91	0.0021
HBAAS (pmol/mg protein)	45.0	39.0–52.4	42.0	36.0–50.0	95	48.0	42.0–57.0	91	0.0003
PLAAS (pmol/mg protein)	20.0	18.0–23.0	19.0	18.0–19.0	98	21.0	19.0–25.0	92	< 0.0001
MDA (pmol/mg protein)	34.8	30.9–40.2	35.4	30.7–40.2	96	33.8	30.9–40.4	92	0.65
Personal $PM_{2.5}$ exposure (µg/m^3)	16.1	10.0–24.5	14.9	9.7–25.0	90	18.8	10.9–24.3	90	0.69
Urban background $PM_{2.5}$ (µg/m^3)[b]	9.2	5.3–14.8	9.1	5.3–15.3	83	9.7	6.2–12.9	74	0.54
Personal carbon black (10^{-6}/m)	8.1	5.0–13.2	8.1	4.2–13.0	92	8.1	5.4–13.3	85	0.55

Q25–Q75, 25th–75th quartile.
[a]The *p*-value for the difference between men and women was calculated by mixed-model regression, with sex as the predictor and subject number nested in sex as random factor. [b]The urban background monitoring station was placed on a roof of a building on the Copenhagen University campus site.

account for factors that could possibly lead to an (within-subject) inherent basis concentration in the dependent variable that was not included in the model. A backward selection was applied, and in the final model only significant factors were included. The dependent variables were logarithmically transformed to obtain variance homogeneity and normal distribution of the residuals. The models are therefore not linear in the original scale, and model estimates represent slopes in the logarithmic analysis. To calculate the predictive value of an X unit increase in the predictors, we used the following formula: [exp(model estimate × X) –1] × 100.

We used similar models to test for associations between the exposure markers, with the natural logarithm of personal $PM_{2.5}$ exposure as dependent variable and personal carbon black exposure and background $PM_{2.5}$ concentration as predictors. Subject number was included as random factor.

Results

Descriptive statistics. Results on hematologic parameters, oxidation products, and exposure markers are shown overall and by sex in Table 1. The hematologic parameters and the protein oxidation products were significantly different in men and women ($p < 0.005$), with RBC and hemoglobin being highest in men and platelets, fibrinogen, HBGGS, HBAAS, and PLAAS being highest in women. There were no significant sex differences in MDA concentrations or in any of the three exposure markers: personal $PM_{2.5}$ exposure, personal carbon black exposure, or background $PM_{2.5}$ concentration.

Relationship between exposure markers and the oxidation products. The relationships between concentrations of PLAAS and personal exposure to $PM_{2.5}$ and carbon black, respectively, are depicted in Figure 1. The personal carbon black exposure and PLAAS concentration was significantly and positively related ($p = 0.009$) with a 4.1% increase in PLAAS per 1×10^{-5}/m increase in personal carbon black exposure (Table 2). The relationship between personal $PM_{2.5}$ exposure and PLAAS was of only borderline significance ($p = 0.061$) with a 1.6% increase in PLAAS per 10 μg/m^3 increase in personal $PM_{2.5}$ exposure (Table 2). PLAAS was not significantly associated with the background $PM_{2.5}$ concentration. Neither HBAAS nor HBGGS was significantly associated with any of the three exposure markers (Table 2). For the three protein oxidation products, season was a significant predictor ($p < 0.05$). Average outdoor temperature was excluded in the backward selection.

In the MDA model the estimates for personal $PM_{2.5}$ exposure were significantly different in women and men ($p = 0.015$), with an increase of 3.7% in MDA concentration per 10 μg/m^3 increase in personal $PM_{2.5}$ exposure in women and no significant relationship for the men. Season and average outdoor temperature were excluded from the model due to insignificance. There were no associations between MDA and personal carbon black exposure or background $PM_{2.5}$ concentration (Table 2).

Relationship between exposure markers and hematologic parameters. The relationship between the personal exposure to $PM_{2.5}$ and the concentrations of RBC and hemoglobin in men and women is depicted in Figure 2. The interaction between sex and personal $PM_{2.5}$ exposure was significant for both the RBC model ($p = 0.048$) and for the hemoglobin model ($p = 0.019$). For the RBC model there was a significant positive association with personal $PM_{2.5}$ exposure in women ($p = 0.009$) with a 2.3% increase in RBC per 10 μg/m^3 increase in personal $PM_{2.5}$ exposure, whereas there was no significant association with personal $PM_{2.5}$ exposure in men (Table 3). Similar results were obtained in the hemoglobin model, with an increase of 2.6% in hemoglobin per 10 μg/m^3 increase in personal $PM_{2.5}$ exposure in women ($p = 0.003$) and no association in men (Table 3). In both models season and average outdoor temperature were removed from the initial model due to insignificance. No relationships could be distinguished with either RBC or hemoglobin when including personal carbon black exposure or background $PM_{2.5}$ concentration instead of personal $PM_{2.5}$ exposure in the model (Table 2). Platelet counts and fibrinogen concentration were not significantly associated with the season, the average outdoor temperature, or with the exposure markers (Table 2).

Relationship among the three exposure markers. The background $PM_{2.5}$ concentration was a predictor of personal $PM_{2.5}$ exposure ($p = 0.03$) with an increase of 12% in personal exposure per 10 μg/m^3 increase in background $PM_{2.5}$. The personal carbon black exposure was also a predictor of personal $PM_{2.5}$ ($p < 0.0001$) with an increase of 30% in personal $PM_{2.5}$ exposure per 1×10^{-5}/m increase in personal carbon black exposure.

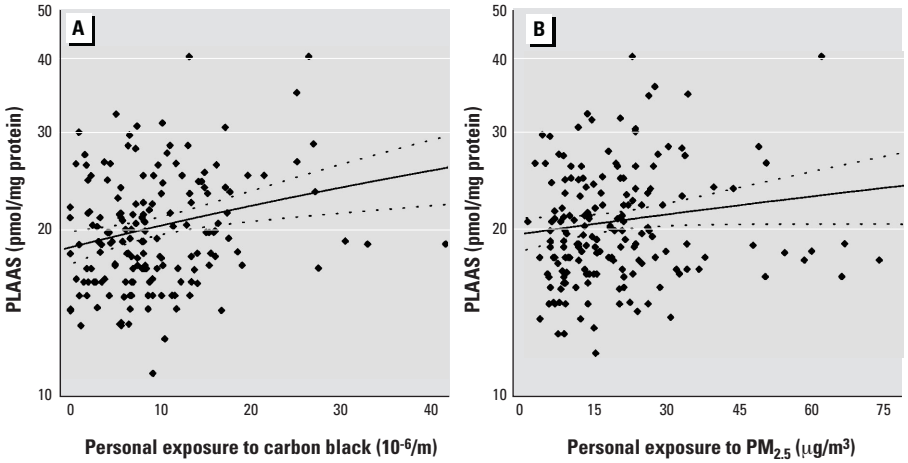

Figure 1. Relationships between 2-aminoadipic semialdehyde in plasma proteins and the personal exposure to (*A*) carbon black and (*B*) $PM_{2.5}$, respectively. The PLAAS values are adjusted with respect to season and sex.

Table 2. The relationship between the exposure markers and the biomarkers.

	Personal exposure to $PM_{2.5}$ (μg/m^3)		Personal exposure to carbon black (10^{-6}/m)		Background concentration of $PM_{2.5}$ (μg/m^3)	
Outcome variables	Estimate	*p*-Value	Estimate	*p*-Value	Estimate	*p*-Value
RBC count (× 10^9/g protein)	(Results in Table 3)		0.0003	0.75	0.0008	0.36
Hemoglobin (μmol/g protein)	(Results in Table 3)		0.0004	0.65	0.0005	0.53
Platelet count (× 10^6/g protein)	0.0008	0.37	0.0009	0.51	–0.0008	0.49
Fibrinogen (nmol/g protein)	0.0006	0.69	–0.0027	0.29	0.0004	0.84
PLAAS (pmol/mg protein)	0.0016	0.061	0.0041	0.0009	0.0004	0.76
HBGGS (pmol/mg protein)	0.0001	0.94	0.0024	0.25	–0.0020	0.39
HBAAS (pmol/mg protein)	0.0006	0.64	0.0022	0.20	–0.0021	0.29
MDA (pmol/mg protein)	(Results in Table 3)		0.0018	0.30	0.0012	0.52

Estimate, model estimate. In the mixed regression models, the outcome variable was transformed by the natural logarithm. Sex exposure marker (personal exposure to $PM_{2.5}$ or reflectance, or background $PM_{2.5}$ concentration), campaign number, average outdoor temperature, and the interaction between exposure marker and sex were set as explanatory variables in 24 separate models. Due to insignificance, the interaction between the exposure marker and sex was excluded from all models, except for three models (described in "Results"): the relationship between *a*) RBC count and personal exposure to $PM_{2.5}$, *b*) hemoglobin and personal exposure to $PM_{2.5}$, and *c*) MDA and personal exposure to $PM_{2.5}$. Average outdoor temperature was excluded from all models due to insignificance.

Discussion

The design of this study, with repeated measurements and inclusion of both men and women, caused the variability in the measured blood components to be determined mainly by differences across the individual subjects and across sex. However, after controlling for differences across subjects and sex, we found significant associations between particulate air pollution and biomarkers of oxidative stress as well as hematologic factors. These associations suggest that personal exposure to fine particles in ambient and/or indoor air can lead to changes and damage to several components of the blood.

There is accumulating evidence that particles are capable of inducing oxidative stress, shown *in vitro* (Prahalad et al. 2001) and *in vivo* (Han et al. 2001). In most of these experiments the particle exposure doses used are many times higher than the exposures seen in this study, and the oxidative markers reflect damage that is quickly removed. We found a significant relationship between the personal carbon black exposure and PLAAS as well as a borderline significant association between personal $PM_{2.5}$ exposure and protein oxidation in plasma (PLAAS). Aminoadipic semialdehyde (AAS) is an oxidation product of lysine and reflects changes in protein damage over a few days up to several weeks (Young et al. 1999, 2002). The results are in agreement with an earlier study conducted on Copenhagen bus drivers, which found the concentration of PLAAS to be significantly higher in bus drivers from central Copenhagen compared with bus drivers from rural/suburban areas (Autrup et al. 1999). Although concentrations of PLAAS in blood can also be influenced by a number of other factors such as diet and age (Daneshvar et al. 1997; Young et al. 1999), this indicates that, even at low concentrations, exposure to particles can cause an increased oxidative stress in the peripheral blood.

Carbon black stems primarily from incomplete combustion, which usually contains toxic substances. Consequently, it has been suggested that carbon black may be a better indicator of the health effect of fine particles than the weight measurement (Horvath 1996; Muir and Laxen 1995). The method responds mainly to particles in the sub-micrometer range, which to a large extent will be ultrafine particles. The association with PLAAS was stronger for the personal carbon black exposure than for $PM_{2.5}$, which could indicate that the ultrafine fraction has the strongest influence on the oxidation of plasma proteins. In recent years several studies have investigated the relationship between personal exposure and indoor–outdoor monitoring of $PM_{2.5}$ or PM_{10}, and many of these studies, including this study, find only weak or no relationship between personal exposure and outdoor concentrations (Koistinen et al. 2001; Ozkaynak et al. 1996; Sorensen M. Unpublished data). This could indicate that measurements of personal exposure include particle fractions that are not included in the background $PM_{2.5}$ concentration and that these fractions are capable of inducing oxidative stress and other adverse health effects.

The personal $PM_{2.5}$ exposure was significantly related to lipid peroxidation measured as MDA in women only. A possible explanation may be that the women in this study were exposed to higher concentrations of $PM_{2.5}$ than were men (Table 1). However, the difference in the median values of personal $PM_{2.5}$ exposure was small (3.9 μg/m^3) and insignificant, and this is most likely not the only explanation. We also found an increase in RBC and hemoglobin in women as a result of the personal $PM_{2.5}$ exposure (Table 3). The production of RBC is controlled by erythropoietin secreted into the

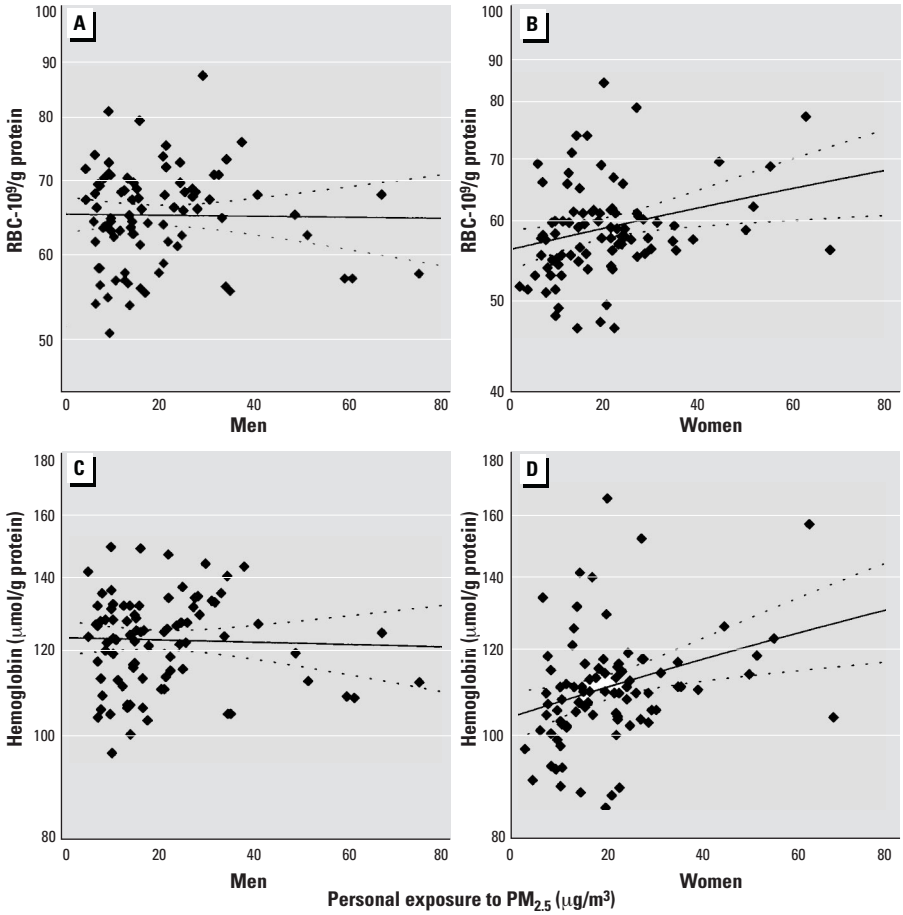

Figure 2. Relationships between counts of red blood cells in (*A*) men and (*B*) women and hemoglobin concentrations in (*C*) men and (*D*) women and the personal exposure to $PM_{2.5}$.

Table 3. The association between the personal $PM_{2.5}$ exposure and red blood cell count (RBC) and hemoglobin, in men and women.

	Model estimate	SE	Increase in dependent variable per increase personal $PM_{2.5}$	95% CI	*p*-Value[a]
RBC					
Personal $PM_{2.5}$ exposure (μg/m^3) × sex					0.048
Men	0.0000	0.0008	0%/10 μg/m^3	−1.6–1.6	0.985
Women	0.0023	0.0009	2.3%/10 μg/m^3	0.5–4.1	0.009
Hemoglobin					
Personal $PM_{2.5}$ exposure (μg/m^3) × sex					0.019
Men	–0.0001	0.0008	0.0%/10 μg/m^3	−1.7–1.5	0.866
Women	0.0026	0.0009	2.6%/ 10 μg/m^3	0.8–4.5	0.003

[a]In the initial mixed regression models the logarithm of RBC × 10^9/g protein and the logarithm of hemoglobin (μmol/g protein) were set as outcome variable. Sex, personal exposure to $PM_{2.5}$, campaign number, average outdoor temperature, and the interaction between the personal exposure to $PM_{2.5}$ and sex were set as explanatory variables. The interaction between the personal exposure to $PM_{2.5}$ and sex was found to be a significant predictor, whereas campaign number and average outdoor temperature were excluded from both models due to insignificance (n = 173 for both models).

blood by cells in the kidneys and, to a lesser extent, the liver. Erythropoietin synthesis is induced by the transcription factor hypoxia-inducible factor 1 (HIF-1). Besides induction by hypoxia, HIF-1 has been induced *in vivo* and *in vitro* by the transition metals cobalt, nickel, and manganese (Chandel et al. 2000; Jasmin and Solymoss 1975; Goldberg et al. 1988). We therefore suggest that the observed increase in RBC concentrations is caused by an HIF-1 induction of erythropoietin, caused by transition metals on the surface of particulate matter. An increase in RBC would cause an increase in blood viscosity, which is a risk factor for cardiovascular events (Donaldson et al. 2001; Lowe et al. 1997). The blood viscosity is determined by several factors besides the RBC concentration, such as plasma viscosity and RBC aggregation. Another study found an increase in plasma viscosity of exposed subjects during air pollution episodes as measured by total suspended particulates, sulfur dioxide, and carbon monoxide (Peters et al. 1997). Peters et al. (1997) found that the increase in plasma viscosity was higher in women than in men for the three exposure markers TSP, SO_2 and CO (when treated as continuous variables). The difference was largest for CO, where only the group of women showed significant odd ratios. This suggests a difference in response to particulate air pollution in women and men. The baseline RBC concentrations is normally higher in men than in women because of the higher concentrations of testosterone in men, which stimulates erythropoietin release, and because of loss of blood during menstruation in women. However, we do not expect that differences in the menstrual cycle can explain the associations seen in this study, as the time of participation was chosen at random, thereby excluding potential systematic errors. This could make the capacity to react to toxicologic influences higher for women than for men.

The present data and the increases in plasma viscosity reported during air pollution episodes (Peters et al. 1997) contrast with a recent study that found negative correlations between estimated personal PM_{10} exposure and hemoglobin concentrations, RBCs, packed cell volume, platelets, fibrinogen, and factor VII in both sexes (Seaton et al. 1999). The offered explanation for those findings was sequestration of RBCs in the peripheral vessels due to particle-induced alteration in adhesive properties. However, in that study the personal exposure was estimated using a mathematical model based on activity diaries and comparative measurements of ambient PM_{10} at multiple sites, and the subjects were all > 60 years old. A study with controlled exposure of human volunteers with a mean age on 24 years found an increase in platelet and neutrophil concentrations in peripheral blood after 1 hr of exposure to 300 $\mu g/m^3$ PM_{10} (generated from an idling diesel engine) in an exposure chamber (Salvi et al. 1999). In addition, several studies have shown positive associations between plasma fibrinogen concentrations and particulate matter (Ghio et al. 2000; Pekkanen et al. 2000). Thus, the effect of age on the response to particle exposure may be significant. This effect needs to be confirmed by further studies.

This study was carried out in a relatively clean environment, with a mean personal $PM_{2.5}$ exposure of 16.1 $\mu g/m^3$. It is uncertain whether the results translate to locations where the personal exposure is higher. However, the dose–response curves presented in Figures 1 and 2 show no signs of deviating from a possible linearity at higher doses.

In conclusion, the associations between particulate exposure and oxidative damage markers and RBCs suggest that particulate air pollution has measurable effects in the blood compartment. Such oxidative stress may be involved in the atherosclerotic process, and increased RBC may affect blood viscosity, suggesting a possible mechanistic relationship with cardiovascular disease. Moreover, the associations were confined to the personal exposure, whereas ambient background concentrations were poorly related to the investigated biomarkers.

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